Qualitative screening of 116 veterinary drugs in feed by liquid chromatography-high resolution mass spectrometry. Potential application to quantitative analysis

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ABSTRACT

Veterinarian and human pharmaceuticals may be intentionally added to animal feed to enhance animal production. Monitoring these substances is necessary for protecting the consumers. In this work, a screening method covering 116 human and veterinary drugs has been developed and validated in five types of animal feed at 0.02 and 0.2 mg kg-1. After a simple extraction with acetonitrile (1% formic acid) and subsequent ten-fold dilution with water, the sample extracts were analyzed by ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS). Nearly all compounds tested were detected at 0.02 mg kg-1, based on the presence of the accurate-mass protonated molecule. The reliable identification of the compound using a second accurate-mass (fragment) ion was however more problematic at this level due to the lower abundance of the second ion in the mass spectra. In a subsequent step, the procedure was applied to 22 feed samples, where compounds as trimethoprim, robenidine, or α- and β-nandrolone were detected and identified. The potential applicability of the method to quantitative analysis of the compounds detected in the samples was also evaluated.

Keywords

Veterinary drugs; animal feed; liquid chromatography; time-of-flight mass spectrometry; screening; qualitative validation.

1. INTRODUCTION

Over the last decades, livestock production has increased notably, mainly due to intensive farming. Veterinary drugs have been extensively used in animal husbandry, both for prophylactic and therapeutic purposes (Lopes, De Freitas Passos, De Alkimim Filho, Vargas, Augusti & Augusti, 2012). It is estimated that 6051 tons of various active substances are used as veterinarian medicines in the European Union to enhance animal production (Kools, Moltmann & Knacker, 2008). Human pharmaceuticals (especially antibiotics) can also be added to animal feed, because of their commercial availability and low cost. In this context, pharmaceutical dosing must be carefully monitored to achieve a compromise between the agronomic results and the negative environmental and sanitary consequences of releasing these drugs to agro ecosystems (Granados-Chinchilla, Sánchez, García & Rodríguez, 2012). The control of these substances is also necessary for protecting the consumers. Animal feeds must have the required quality and be appropriate from a nutritional point of view. They must be safe, i.e., free from contaminants and residues in general, and from residues of veterinary drugs in particular. The case of antimicrobials is of particular concern, as they might provoke allergies and contribute to the development of resistant bacterial strains if they reach the food chain (Borràs et al., 2011).

Some substances are banned, while others are authorized as long as their concentrations in food of animal origin remain below certain established limits. Thus, Directive 2002/32/EC regulated the measures on undesirable substances in animal feed (European Commission, 2002a). Regulation (EC) 1831/2003 banned the use of all antibiotics other than coccidiostatics and histomonostats as feed additives from 1 January 2006 (European Commission, 2003). Recently, Directive 2009/8/EC

established maximum levels of unavoidable carry-over for these compounds in non-target feed (European Commission, 2009).

Drugs can reach feeds in three ways: authorized drugs (for therapeutic and prophylactic purposes), unauthorized drugs (as grow promoters to increase yield) and unintentional (as a result of the so-called cross-contamination) (Borrás, Ríos-Kristjánsson, Companyõ & Prat, 2012). Although several different analytical methods based on liquid chromatography coupled to fluorescence or ultraviolet detection have been developed, the most recent methodology relies on mass spectrometry (Capitan-Vallvey, Ariza, Checa & Navas, 2007, Kot-Wasik & Wasik, 2005) or, preferably, tandem mass spectrometry detection with triple quadrupole (Boscher, Guignard, Pellet, Hoffmann & Bohn, 2010, Cronly et al., 2010, De Baere & De Backer, 2007, Van Holthoon, Mulder, Van Bennekom, Heskamp, Zuidema & Van Rhijn, 2010, Vincent, Chedin, Yasar & von Holst, 2008) or ion trap analyzers (Kantiani, Farré, Freixiedas & Barceló, 2010, Xu et al., 2011), because of the high selectivity and sensitivity provided by this technique. However, most of methods developed until now deal with a limited number of compounds, generally belonging to the same family. Among the most studied are the quinolones antimicrobials (Borrás, Ríos-Kristjánsson, Companyõ & Prat, 2012, Boscher, Guignard, Pellet, Hoffmann & Bohn, 2010, Xu et al., 2011), sulfonamides (Kantiani, Farré, Freixiedas & Barceló, 2010, Lopes, De Freitas Passos, De Alkimim Filho, Vargas, Augusti & Augusti, 2012), macrolides (Boscher, Guignard, Pellet, Hoffmann & Bohn, 2010), β-lactams (Boscher, Guignard, Pellet, Hoffmann & Bohn, 2010, Kantiani, Farré, Freixiedas & Barceló, 2010, Van Holthoon, Mulder, Van Bennekom, Heskamp, Zuidema & Van Rhijn, 2010) or tetracyclines (Boscher, Guignard, Pellet, Hoffmann & Bohn, 2010, Granados-Chinchilla, Sánchez, García & Rodríguez, 2012). In these cases, specific sample treatments are normally applied.

The large number of available drugs has caused an increase in the number of analytes to be monitored. Under this situation, it is advisable to perform sample extractions as generic as possible in order to widen the scope of the method and to include as many analytes as possible. For large screening purposes, an alternative to MS/MS is the application of full scan techniques based on high resolution mass spectrometry (HRMS), using QTOF (Deng et al., 2011, Nácher-Mestre, Ibáñez, Serrano, Pérez-Sánchez & Hernández, 2013, van der Heeft, Bolck, Beumer, Nijrolder, Stolker & Nielen, 2009, Villar-Pulido, Gilbert-López, García-Reyes, Martos & Molina-Díaz, 2011) or Orbitrap (Kaufmann, Butcher, Maden, Walker & Widmer, 2011, van der Heeft, Bolck, Beumer, Nijrolder, Stolker & Nielen, 2009) analyzers, which have opened new possibilities for analysis of many different organic contaminants/residues in matrices like milk (Freitas, Paim & de Souza e Silva, 2013, Stolker et al., 2008) urine (León, Roca, Igualada, Martins, Pastor & Yusá, 2012), water (Diaz, Ibáñez, Sancho & Hernández, 2013) or feed (Aguilera-Luiz, Romero-González, Plaza-Bolaños, Martínez Vidal & Garrido Frenich, 2013, Martínez-Villalba, Vaclavik, Moyano, Galceran & Hajslova, 2013, Nácher-Mestre, Ibáñez, Serrano, Pérez-Sánchez & Hernández, 2013). As illustrative examples, Martínez et al. (Martínez-Villalba, Vaclavik, Moyano, Galceran & Hajslova, 2013) and Nácher et al. (Nácher-Mestre, Ibáñez, Serrano, Pérez-Sánchez & Hernández, 2013) carried out a qualitative validation for chicken and fish feed, respectively, using HRMS. Aguilera et al. (Aguilera-Luiz, Romero-González, Plaza-Bolaños, Martínez Vidal & Garrido Frenich, 2013), made the validation for around 60 veterinary drugs and 150 pesticides in chicken, hen, rabbit and horse feed.

The objective of this work is to investigate the potential of UHPLC coupled to hybrid analyzer QTOF MS for large screening (i.e. detection and identification of the compound detected) of human and veterinary drugs in different animal feeds (bovine,

rabbit, poultry, goat and pork). This analyzer allows to work under MS^E mode, which allows the simultaneous acquisition of accurate-mass full-spectrum acquisition data at low (LE) and high (HE) collision energy. MS^E mode provides useful information on the parent molecule (commonly the (de)protonated molecule, in the LE function, as this is the main ion observed in electrospray source under negative or positive ionization mode), and on the main fragment ions (commonly in the HE function, where fragmentation is promoted) (Diaz, Ibáñez, Sancho & Hernández, 2013; Hernández, Bijlsma, Sancho, Díaz & Ibáñez, 2011). A subset of around 120 compounds from different chemical families has been selected for evaluation of the screening methodology. Once validated, the screening has been widened to search for around 530 additional pharmaceuticals, which reference standards were unavailable in our laboratory, and applied to 22 commercial feed samples to test its applicability. The possibilities to perform quantitative analysis for those positive pharmaceuticals found in animal feeds using the same instrument have been also evaluated.

2. EXPERIMENTAL

2.1. Reagents and chemicals

Reference compounds (**Table S1)** were purchased from Fort Dodge Veterinaria (Gerona, Spain), Vetoquinol Industrial (Madrid, Spain), Aventis Pharma (Madrid, Spain), Sigma Aldrich (St Louis, MO, USA), Cerilliant (Round Rock, TX, USA), Dr. Ehrenstorfer (Augsburg, Germany), Riedel-de Haën (Seelze, Germany), National Measurement Institute (Pymble, Australia), Witega (Berlin, Germany), and Fluka (Buchs, Switzerland). All reference materials had purities higher than 98% (w/w), except for marbofloxacin and pefloxacin, which had purities higher than 93%.

Isotopically labelled internal standards (ILIS) fenilbutazone-d_{10}, robenidine-d_8, 4,4'dinitrocarbanilide-d_8 (DNC-d_8), amphetamine-d_6, benzoylecgonine-d_3 and carbamazepine epoxide-d_{10} were obtained from Cerilliant, and CDN Isotopes (Quebec, Canada).

HPLC-grade methanol (MeOH), acetonitrile (ACN) and sodium hydroxide (>99%) were purchased from ScharLab (Barcelona, Spain). Formic acid (HCOOH) (>98% w/w) was obtained from Fluka. Leucine enkephalin was purchased from Sigma Aldrich. HPLC-grade water was obtained from deionized water passed through a Milli-Q Gradient A10 (18.2 MΩ cm) water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

An Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) was interfaced to a QTOF mass spectrometer (QTOF Xevo G2, Waters Micromass, Manchester, UK) using an orthogonal Z-spray electrospray interface. The LC separation was performed using Acquity UPLC BEH C18 1.7 µm particle size analytical column of 100 x 2.1 mm (from Waters), at a flow rate of 0.3 mL min-1. The mobile phases used were A H_2O and B MeOH, both with 0.01% (v/v) HCOOH. The percentage of MeOH was linearly increased as follows: 0 min, 10%; 14 min, 90%; 16 min, 90% and 16.01 min, 10%. The total run time was 18 min. The injection volume was 50 µL. Nitrogen (Praxair, Valencia, Spain) was used as both the drying gas and the nebulising gas. The desolvation gas flow rate was set at 1000 L h-1. The resolution of the TOF mass spectrometer was ~20 000 at full width half maximum (FWHM) at *m/z* 556. MS data were acquired over a *m/z* range of 50–1200 in a scan time of 0.3 s. Capillary voltages of 0.7 kV and -1.7 kV were used in positive and negative ionization modes, respectively. A cone voltage of 25 V was applied. The collision gas was argon

(99.995%, Praxair). The interface temperature was set to 650 ºC and the source temperature to 130 ºC. The column temperature was set to 40 ºC and the samples to 5 ºC. For MS^E experiments, two acquisition functions with different collision energies were created: the low-energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 15 to 40 eV. The same cone voltage (25 V) and collision energy ramp was used for additional MS/MS experiments.

Calibration of the mass-axis from *m/z* 50 to 1200 was conducted daily with a 1:1 mixture of 0.05M NaOH/5% (v/v) HCOOH diluted (1:25) with water/ACN (20:80 v/v).

For automated accurate mass measurement, the lock-spray probe was employed, using as lockmass leucine enkephalin (2 mg L-1) in ACN/water (50/50) at 0.1% HCOOH, pumped at 20 µL min-1 through the lock-spray needle. The leucine enkephalin $[M+H]^+$ ion (*m/z* 556.2771) and its fragment ion (*m/z* 278.1141) for positive ionization mode, and $[M-H]^-$ ion (*m/z* 554.2615) and its fragment ion (*m/z* 236.1035) for negative ionization, were used for recalibrating the mass axis and to ensure a robust accurate mass measurement over time.

The data station operating software was MassLynx version 4.1 (Waters).

2.3. Feed samples

A total of 10 different feed samples were used for method validation (2 bovine, 2 rabbit, 2 poultry, 2 goat and 2 pork).

In a subsequent step, the developed procedure was applied to other feed samples to test its applicability. 22 feed samples (12 bovine, 3 rabbit, 2 poultry, 2 goat and 3 pork) were collected in polyethylene high-density bottles from farms located in Spanish Mediterranean area (Valencia and Castellon provinces). Samples were stored at −18 ºC.

Before analysis, samples were thawed at room temperature and triturated with a crusher Super JS from Moulinex (Bagnolet Cedex, France).

2.4. Extraction procedure

Five grams of homogenized feed sample were accurately weighed (precision 0.1 mg) directly in centrifuge tubes (50 mL) and extracted with 10 mL of acetonitrile 1% HCOOH, using a vortex for 1 minute. Then, the mixture was mechanically shaken end-over-end for 1 hour. After that, samples were sonicated (15 minutes) and centrifuged at 4500 r.p.m for 10 min. A 2-mL aliquot of supernatant was transferred to an Eppendorf tube, and a second centrifugation was performed at 12000 r.p.m (12074 x g) for 10 min. Finally, the extract was ten-fold diluted with Milli-Q water (100 µL extract + 900 µL water) and injected in the system. No microfiltration was made to avoid potential losses of compounds in this step.

2.5. Qualitative validation protocol

In this work, method validation was performed following the strategy described in the literature (Diaz, Ibáñez, Sancho & Hernández, 2013, Nácher-Mestre, Ibáñez, Serrano, Pérez-Sánchez & Hernández, 2013).

Ten different animal feeds (five types of matrices, two feed samples for each matrix) were spiked with a mix solution of the test analytes in methanol at two levels, 0.02 mg kg-1 and 0.2 mg kg-1, let stand overnight, and analyzed together with their non-spiked samples (blanks). The final concentrations in the ten-fold diluted extracts were 1 and 10 µg L-1, respectively.

The screening detection limit (SDL) and the limit of identification (LOI) were investigated as the main validation parameters to estimate the threshold concentration at which detection and identification become reliable, respectively. The SDL was

established as the lowest concentration level tested for which a compound was detected in all samples, using the most abundant ion (typically, the (de)protonated molecule) measured at its exact mass (mass error lower than ±2 mDa) and at the expected retention time (±2.5% RT deviation tolerance). The LOI was established as the lowest concentration tested for which a compound was satisfactorily identified in all spiked samples. The identification criterion was the presence of, at least, two *m/z* ions in either the LE or HE function at the expected retention time measured at their exact mass.

The terms SDL and LOI would be equivalent to the definition of "screening target concentration" and "detection capability", respectively, provided by the CRL´s 2010 guideline (CRLs 2010).

3. RESULTS AND DISCUSSION

3.1. Database building

A large number of pharmaceuticals, which could be potentially present in animal feed, were selected as target compounds, with *m/z* values ranging from 137.0239 ($[M-H]^-$ ion of salicylic acid) to 934.5739 ($[M+H]^+$ ion of maduramicine). For those pharmaceuticals which reference standard was available in our lab (116 compounds), empirical data was obtained after injection into the UHPLC-QTOF MS, following the working conditions reported in Diaz et al, 2011 (Díaz, Ibáñez, Sancho & Hernández, 2011). These compounds were afterwards used in the validation experiments. **Table S1** shows the compound name, ionization mode, retention time (min.) and exact mass for (de)protonated compounds, and the elemental compositions for their main fragment ions. 93 out of 116 analytes were detected in positive ionization mode, whereas 9 were in negative mode; 14 compounds were detected in both modes. For the remaining

compounds, which standards were unavailable (530 approx.), information on elemental composition was included in the database, for future screening in real samples. The database included anabolic substances (such as thyrostatic compounds, stilbenes, stilbenes derivatives, 17-β-estradiol and ester derivatives) as well as β-agonists, prohibited according to (European Commission, 2008). Compounds with maximum levels established (coccidiostatics and histomonostats) (European Commission, 2002a) and other veterinarian and human pharmaceuticals not regulated, mainly antimicrobials, were also included.

3.2. Qualitative validation results

Five different feed matrices were tested in method validation: bovine, rabbit, poultry, goat and pork. Two samples of each type were spiked with the mix of 116 pharmaceuticals at two concentration levels (0.02 and 0.2 mg kg-1). These levels were selected accordingly with the feed regulation (Bruni & Ferreira, 2008). **Table S2** shows the SDL and LOI obtained for each analyte in each matrix. A summary of this table is also shown in **Figure S1**. As it can be seen, around 75% of compounds could be detected at 0.2 mg kg-1 (see accumulated, **Figure S1**) while the percentage of detection decreased down to 40% at 0.02 mg kg-1. It is noteworthy the great differences observed between the matrices studied. As shown in **Figure S2**, significant differences in the signal response (e.g. in sensitivity) were found between standards in solvent and in the five feed samples (all at a concentration of 0.2 mg kg-1), as illustrated for six of the compounds investigated. This made that, although around between 90% of the compounds were detected in each matrix individually at 0.2 mg kg-1, only 75% could be fully validated at this level, i.e. only 75% were detected in all matrices. The highest number of compounds detected corresponded to poultry and pork feeds (around 70 pharmaceuticals). On the contrary, bovine, rabbit and goat feeds appeared as the most

problematic matrices, which may be considered more complex than others, likely due to their different fatty content (Aguilera-Luiz, Romero-González, Plaza-Bolaños, Martínez Vidal & Garrido Frenich, 2013). Nine pharmaceuticals (diethylstilbestrol, 16-β-hydroxystanozolol, amoxicillin, cefaclor, cefadroxil, cephalexin, abamectine, doramectine and omeprazole) were not detected in any of the spiked samples. These compounds might not be stable under the extraction conditions assayed or might be highly affected by matrix effects.

Overall, the reliable identification using two accurate-mass ions was feasible for 55% of compounds at 0.2 mg kg-1. This value drastically decreased down to 10% at 0.02 mg kg-1, showing the great difficulties to obtain a second ion at low analyte concentration in this type of matrices. Regarding poultry and pork feeds around 40% of compounds could be identified at the lower level; yielding more than 70% of the pharmaceuticals identified at 0.2 mg kg-1. The apparently more complex goat, rabbit and bovine feed matrices allowed the identification of around 60% of the compounds analyzed at the 0.2 mg kg-1 level. Concentrations higher than 0.2 mg kg-1 were not tested. Surely, most of compounds which were detected but could not be identified at 0.2 mg kg-1 because of the low sensitivity for its fragment ion might be identified at higher concentration levels.

In this work, typically the (de)protonated molecule and at least one collision induced dissociation (CID) fragment ion, in either, the LE or HE functions, were used for detection and identification, respectively. However, several compounds presented poor or none fragmentation as result of the compromise collision energy ramp applied. This was the case of ivermectin or ipromidazole, amongst others. In those cases, when fragment ions were not observed, only SDL could be set-up (12 out of 116 compounds).

Table 1 shows the total SDL for the 10 samples under study (i.e. SDL means detected in all 10 samples analyzed), and illustrates the applicability of the screening for detection of veterinary drugs..

3.3. Application to routine samples

A total of twenty two feed samples (12 bovine, 3 rabbit, 2 poultry, 2 goat and 3 pork) were analyzed following the developed procedure in order to evaluate its applicability. Up to 11 compounds were detected and properly identified. **Table 2** shows a summary of the results obtained.

The most detected compounds were the salicylic acid, active metabolite of anti-inflammatory acetylsalicylic acid (aspirin), and the antimicrobial trimethoprim, which were found in 50% of the samples analyzed. As an illustrative example, **Figure S3** shows the HE spectra of a reference standard of trimethoprim and a positive poultry feed sample. As it can be seen, both spectra perfectly matched, sharing up to 8 main fragment ions with mass errors lower than 2 mDa, at the expected retention time (3.6 min). **Figure 1** shows another example, the detection and identification of the antibiotic lincomycin in a pork feed sample. In this case, the ions corresponding to the protonated molecule and to its unique fragment ion (at *m/z* 407.2216 and *m/z* 126.1283, respectively) at the expected retention time were observed. In addition to the presence of the two ions with acceptable mass errors, the intensity ratio between the most abundant ion (i.e. the protonated molecule) and the fragment ion used for confirmation was also calculated obtaining a deviation of 1.3% in relation to the reference standard. This deviation was by far lower than the ± 20% allowed by the EU guidelines for ion ratios between 1 and 2, giving even more reliability to the confirmation process (European Commission, 2002b).

Up to 4 tetracyclines (chlortetracycline, oxytetracycline, tetracycline and doxycycline) were found in several matrices, the highest levels being found in one of the pork feeds particularly oxytetracycline (**Table 2**).

The antibiotic florfenicol was also identified in 2 bovine samples. This antimicrobial is currently indicated for the treatment of bovine respiratory diseases (European Medicines Agency,2009).

The detection of robenidine, a coccidiostat used for the control of protozoal infection, and regulated in the European Commission Directive EC 2009/8 (European Commission, 2009) was also of interest. This compound was detected in one bovine and two rabbit feed samples. **Figure 2** illustrates the identification and quantification of this pharmaceutical in rabbit feed. As it can be seen, not only the protonated molecule but also two fragment ions were observed at the expected retention time. Robenidine-d_8 was used as isotope-labeled internal standard (ILIS) to compensate for matrix affects as well as possible variations in the instrument measurement. The concentration calculated for this sample was 40.4 mg kg-1 (see next section for more details). Although this value by far exceeds the maximum content of 0.7 mg kg-1 established by the Directive (European Commission, 2010), a subsequent Commission Regulation (Commission implementing Regulation (EU) No 532/2011 of 31 May 2011 concerning the authorization of robenidine hydrochloride as a feed additive for rabbits for breeding and rabbits for fattening (holder of authorization Alpharma Belgium BVBA) and amending Regulations (EC) No 2430/1999 and (EC) No 1800/2004) concerning the authorization of robenidine hydrochloride as a feed additive for rabbits for breeding and rabbits for fattening, establishes the maximum content in 66 mg of active substance kg-1 of complete feeding stuff, until June 2021 (European Commission, 2011).

The most remarkable was the detection of the steroids α-nandrolone and β-nandrolone, found in 7 and 1 out of 22 feed samples analyzed, respectively (**Table 2**). These compounds were banned by Directive 2008/97/EC European Parliament and of the Council of 19 November 2008 amending Council Directive 96/22/EC concerning the prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of beta-agonists (European Commission, 2008). The identification of the hormone α-nandrolone in bovine feed is shown in **Figure 3,** where the protonated molecule and two fragment ions were detected in the LE function. To support the confirmation of its identity, the intensity ratio was calculated too, obtaining a deviation of -44% for the main fragment ion (F1) which is lower than ± 50% allowed by the EU guidelines for ion ratios ≥ 10 (European Commission, 2002b).

Although the LOI could not be established to be 0.2 mg kg-1 for some compounds (i.e., the compound could not be identified at this level in spiked samples), they were however identified in the feed samples analyzed. This means that their concentration in the samples was above the levels tested for LOI (0.02 and 0.2 mg kg-1). This was the case of chlortetracycline in poultry feed, doxycycline in rabbit and pork feed, tetracycline in poultry and bovine feed, and salicylic acid in poultry, bovine, rabbit and pork feed. With the data available from QTOF MS analysis, the identification of these compounds in the samples was certainly highly reliable.

In case of detecting a compound whose reference standard was not available in the laboratory, its tentative identification would be based on the ions observed (protonated molecule and fragment ions), their compatibility with the chemical structure of the candidate, and on the comparison with those ions reported in the literature (Diaz, Ibáñez, Sancho & Hernández, 2013; Díaz, Ibáñez, Sancho & Hernández, 2012). In the

present work, for all compounds detected in feed the reference standards were all available in our laboratory.

3.4. Evaluation of the applicability to quantitative analysis

Finally, an evaluation of the applicability of the screening to quantitative analysis was made for the compounds found in the feed samples: β-nandrolone, robenidine, chlortetracycline, tetracycline, oxytetracycline, doxycycline, lincomycin, trimethoprim, florfenicol and salicylic acid. This investigation was not possible for α-nandrolone due to the lack of reference standard at sufficiently high concentration (a mixture of several standards, at around 10 mg L-1, was only available for this compound as a gift from the Laboratory of Public Health of Valencia).

For this purpose, bovine, poultry and pork feeds were spiked by triplicate at 0.2 and 2 mg kg-1. Those feeds previously analyzed and proven to not contain the analytes were selected as "blanks" to perform recovery experiments (one feed for each matrix by triplicate). In addition to spiked samples, "blank" samples, spiked only with an ILIS mix, were also processed to subtract the responses of possible positive compounds. Quantification was performed using calibration standards in solvent and relative responses to ILIS for matrix effects correction. Unfortunately, robenidine was the only analyte among all tested for which its labeled compound (robenidine-d_8) was available at our laboratory. After several preliminary experiments, for the rest of compounds we selected other available ILIS that seemed to roughly compensate the matrix effect observed (**Table S3**).

The linearity of the method was studied in the range 1-250 µg L-1 for all selected compounds obtaining satisfactory correlation coefficients (greater than 0.99).

Regarding robenidine, satisfactory recoveries (61-93%) and precision (1-6%) were obtained at 0.2 mg kg-1 spiked level for the three matrices, and also at 2 mg kg-1 (recovery 90-98%, precision 3-6%) (**Table S3)**. Nevertheless, for the other compounds, recoveries were not completely satisfactory in all matrices although they mostly varied between 60-120%. Tetracyclines could only be validated in pork feed at the highest level assayed. With few exceptions, RSD were satisfactory (below 15%) except for chlortetracycline in pork, and salicylic acid in bovine and poultry. It seemed that the analyte-labeled ILIS was required to ensure appropriate correction due to the strong matrix effect resulting from the matrix complexity and little sample manipulation. **Figure S2** shows the responses for six pharmaceuticals in solvent and in five feed matrices spiked at 0.2 mg kg-1, illustrating the large signal differences observed between each feed matrix. Matrix effects correction using ILIS other than the labeled analyte was not always assured. Based on the experiments performed, we quantified the compounds detected in the samples only for those matrices previously tested, which recoveries ranged between 60 and 120% with RSD < 15% (see **Table 2**). Although rabbit feed was not tested in recovery experiments, a high robenidine concentration of 40.4 mg kg-1 could be reported due to the satisfactory recovery obtained in other matrices and to the availability of analyte ILIS.

The objective of the present work was to investigate the potential of LC-QTOF MS for screening of veterinary drugs. In the case that this methodology was finally used by reference laboratories and for official purposes, the appropriate guidelines should be taken into account, as for example the one established for community reference laboratories residues (CRLs 2010), or other guidelines applied in related fields (European Commission, 2013).

4. CONCLUSIONS

In this article, a multiclass, wide-scope, and rapid screening based on UHPLC-QTOF MS has been developed for human and veterinary pharmaceuticals in five types of animal feeds (bovine, rabbit, poultry, goat and pork). After extraction of the sample with acidified acetonitrile and direct analysis by QTOF MS, the wide majority of the 116 compounds tested were detected and correctly identified in all feed samples spiked at 0.2 mg kg-1. Detection, based on the presence of the (de)protonated molecule, was also feasible in most cases at the lowest level tested (0.02 mg kg-1), although identification using a second accurate-mass fragment ion, was problematic at this concentration due to the complexity of the sample matrices.

The screening procedure was applied to 22 feed samples, with the result of detecting and correctly identifying several antibiotics, such as florfenicol, robenidine or lincomycin; one of them, robenidine, included in the current legislation. Moreover, two hormones, α- and β- nandrolone, banned by the directive (European Commission, 2008), were also found in some feeds. Trimethoprim and salicylic acid, were the compounds more detected (50%) followed by lincomycin and α-nandrolone (32%).

The strong potential of LC-QTOF MS for wide-scope screening of veterinary drugs in feed has been proven in this work. Although the main applications of QTOF MS are directed towards detection and identification of the compounds, this technique might be also used for quantitative purposes. To explore this possibility, a preliminary work has been made with the compounds that were detected in the previous screening obtaining promising results. The availability of robenidine-d_8 used as ILIS allowed the reliable quantification of robenidine in the samples analyzed, thanks to the satisfactory matrix effects correction, as demonstrated by the satisfactory recoveries and precision obtained at 0.2 and 2 mg kg-1 spiked levels. This compound was found in two rabbit feeds with a

maximum concentration of 40.4 mg kg-1. In the light of the results obtained in this work, a quantitative analysis might be also feasible using LC-QTOF MS, although more work is required to fully explore this possibility. This technique, similarly to the most widely used for quantitative analysis in this field (i.e. LC-MS/MS), is notably affected by matrix effects, and therefore the use of ILIS greatly facilitates an accurate quantification.

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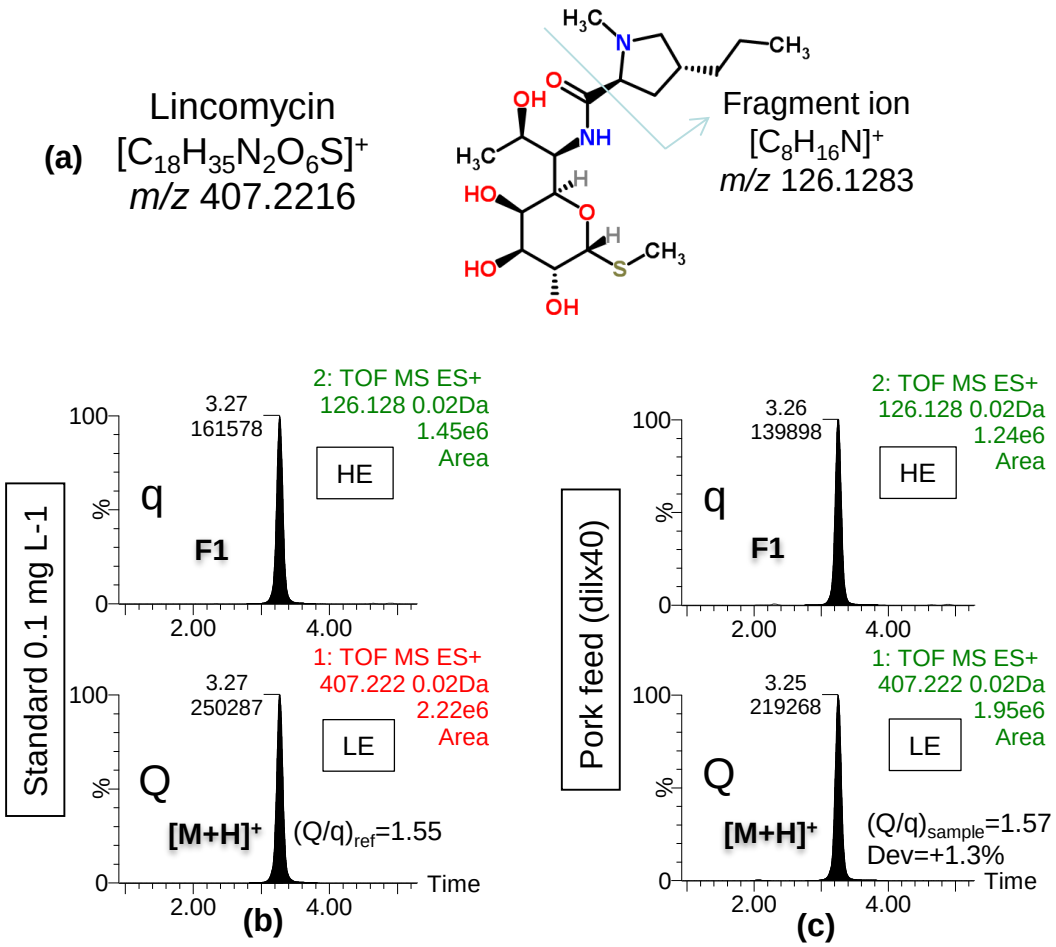
FIGURE CAPTIONS

Figure 1. (a) Lincomycin and its fragment ion structure. nw-XICs corresponding to the protonated molecule at LE and to its fragment ion at HE for (b) 0.1 mg L-1 reference standard and (c) positive pork feed sample.

Figure 2. Positive finding of the coccidiostatic robenidine in a rabbit feed. (a) nw-XICs at 20 mDa mass window for the protonated molecule at LE and its two main fragment ions at HE. (b) Calibration curve obtained using relative responses, with robenidine-d_8 as internal standard.

Figure 3. Positive finding of α-nandrolone in a bovine feed. nw-XICs at 20 mDa mass window for the protonated molecule and two fragment ions at LE for (a) reference standard and (b) bovine feed.



15

16 Figure 1

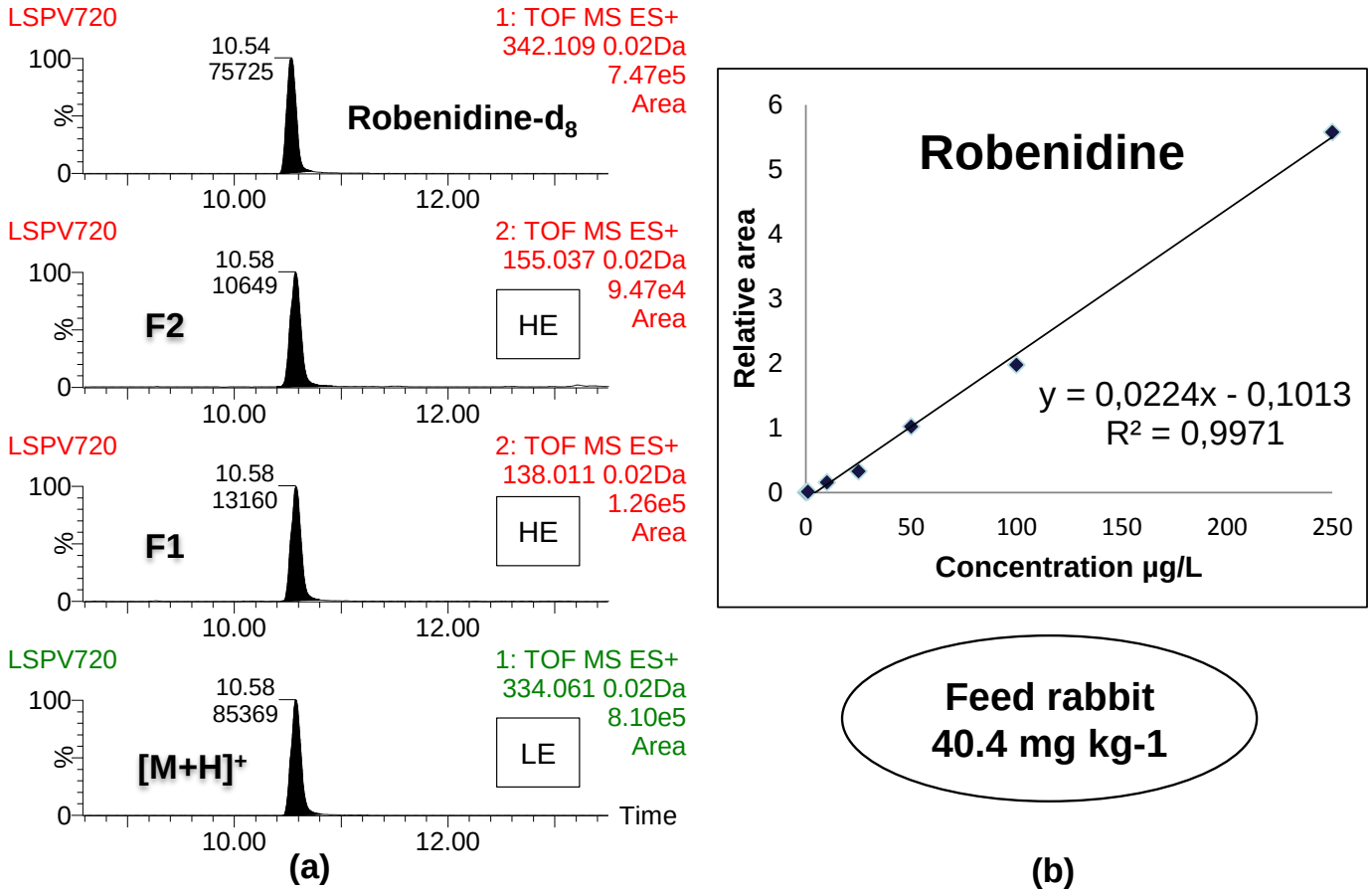

LSPV720
1: TOF MS ES+
342.109 0.02Da
7.47e5
Area
10.54
75725
Robenidine-d8
LSPV720
2: TOF MS ES+
155.037 0.02Da
9.47e4
Area
10.58
10649
F2
HE
LSPV720
2: TOF MS ES+
138.011 0.02Da
1.26e5
Area
10.58
13160
F1
HE
LSPV720
1: TOF MS ES+
334.061 0.02Da
8.10e5
Area
10.58
85369
[M+H]+
LE
100
%
0
10.00
12.00
Time
(a)
Robenidine
Relative area
Concentration µg/L
y = 0,0224x - 0,1013
R² = 0,9971
0
50
100
150
200
250
1
2
3
4
5
6
Feed rabbit
40.4 mg kg-1
(b)

Figure 2

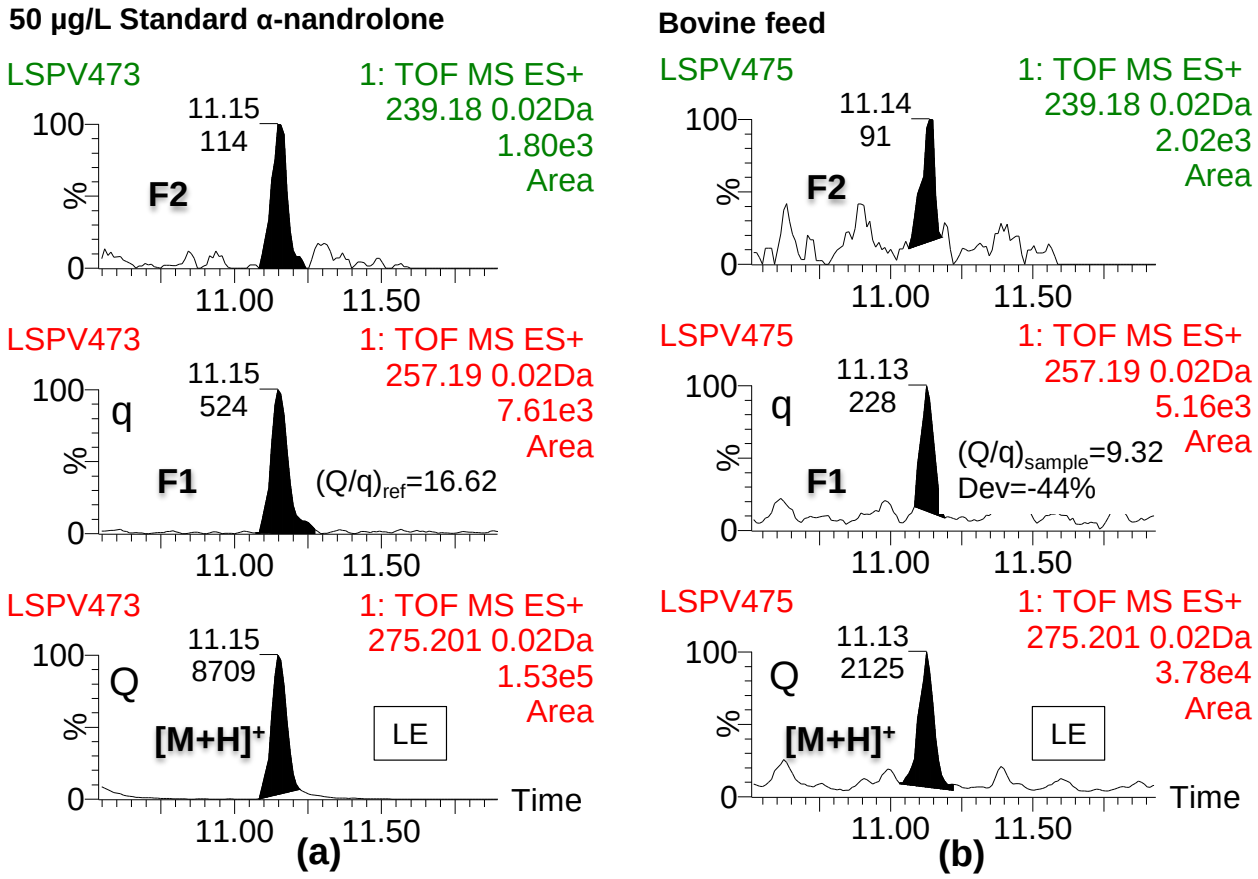

50 µg/L Standard α-nandrolone
LSPV473
1: TOF MS ES+
239.18 0.02Da
1.80e3
Area
11.15
114
F2
100
%
0
11.00
11.50
LSPV473
1: TOF MS ES+
257.19 0.02Da
7.61e3
Area
11.15
524
q
F1
(Q/q)ref=16.62
LSPV473
1: TOF MS ES+
275.201 0.02Da
1.53e5
Area
11.15
8709
Q
[M+H]+
LE
Time
(a)
Bovine feed
LSPV475
1: TOF MS ES+
239.18 0.02Da
2.02e3
Area
11.14
91
F2
LSPV475
1: TOF MS ES+
257.19 0.02Da
5.16e3
Area
11.13
228
q
F1
(Q/q)sample=9.32
Dev=-44%
LSPV475
1: TOF MS ES+
275.201 0.02Da
3.78e4
Area
11.13
2125
Q
[M+H]+
LE
Time
(b)

Figure 3

Table 1. Screening Detection Limit (SDL) and Limit Of Identification (LOI) (both in mg Kg-1) for studied compounds.

Stilbenes	**SDL**	**LOI**
Dienestrol	>0.2	>0.2
Hexestrol	>0.2	>0.2
Steroids	SDL	LOI
Δ1,4-Androstadiene-3,17-dione (Boldione)	>0.2	>0.2
α-Boldenone	0.02	0.2
α-Nandrolone	0.02	>0.2
Androstenediol (AED)	>0.2	>0.2
β-Boldenone	0.2	>0.2
β-Nandrolone	0.2	0.2
Stanozolol	0.02	>0.2
RALs	**SDL**	**LOI**
α-Zeranol	>0.2	>0.2
β-Zeranol	0.2	>0.2
Zearalanone (ZAN)	>0.2	>0.2
β-Agonists	**SDL**	**LOI**
Brombuterol	0.02	0.2
Clenbuterol	0.02	0.2
Clenpenterol	0.02	0.2
Hydroxymethyl clenbuterol	0.02	0.02
Mabuterol	0.02	0.02
Mapenterol	0.02	0.02
Ractopamine	0.02	0.2

Nitromidazoles	**SDL**	**LOI**
Chloramphenicol	0.02	0.2
Dimetridazole (DMZ)	0.02	>0.2
Furaltadone	0.2	>0.2
Hydroxy Dimetridazole (HMMNI)	>0.2	>0.2
Hydroxyipronidazole (IPZOH)	0.02	0.02
Hydroxymetronidazole (MNZOH)	>0.2	>0.2
Ipronidazole (IPZ)	0.2	>0.2
Metronidazole (MNZ)	>0.2	>0.2
Ronidazole (RNZ)	>0.2	>0.2
Amphenicols	**SDL**	**LOI**
Ampicillin	0.2	>0.2
Cefotaxim	0.2	0.2
Ceftriaxone	>0.2	>0.2
Cefuroxime	>0.2	>0.2
Chlortetracycline	>0.2	>0.2
Ciprofloxacin	0.2	0.2
Clarithromycin	0.02	0.02
Cloxacillin	0.2	0.2
Dicloxacillin	0.2	>0.2
Doxycycline	>0.2	>0.2
Enrofloxacin	0.2	0.2
Erythromycin A	0.2	>0.2

Amphenicols (cont.)	**SDL**	**LOI**
Florfenicol	0.2	>0.2
Florfenicol amine	>0.2	>0.2
Flumequine	0.02	0.2
Lincomycin	0.02	0.2
Nalidixic acid	0.02	0.2
Norfloxacin	0.2	0.2
Ofloxacin	0.2	0.2
Oxacillin	0.2	>0.2
Oxolinic acid	0.2	0.2
Oxytetracycline	>0.2	>0.2
Pefloxacin	0.02	0.2
Penicillin G	0.2	0.2
Pipedimic acid	0.2	0.2
Piperacillin	0.2	0.2
Roxythromycin	0.02	0.2
Sarafloxacin	0.2	>0.2
Sulfadoxine	0.02	0.2
Sulfamethoxazole	0.02	0.2
Tetracycline	>0.2	>0.2
Thiamphenicol	0.2	>0.2
Trimethoprim	0.02	0.02

Table 1 (cont). Screening Detection Limit (SDL) and Limit Of Identification (LOI) (both in mg Kg-1) for studied compounds.

Avermectins	**SDL**	**LOI**
Emamectin B1a	0.2	0.2
Eprinomectin	0.2	>0.2
Ivermectin	>0.2	>0.2
Levamisole	0.02	0.2
Moxidectin	>0.2	>0.2
Coccidiostats	**SDL**	**LOI**
Maduramicine	0.02	0.2
Monensin	0.2	0.2
Narasin	0.02	>0.2
Robenidine	0.02	0.2
Salinomycin	0.2	>0.2
Sedatives	**SDL**	**LOI**
Acepromazine	0.02	0.2
Alprazolam	0.02	0.2
Azaperol	0.02	>0.2
Azaperone	0.02	0.2
Carazolol	0.02	0.02
Chlorpromazine	0.02	0.02
Propionilpromazine (combelen)	0.02	0.2

NSAIDs	**SDL**	**LOI**
4-acetylamino-antipyrine	0.02	0.2
4-amino-antipyrine	>0.2	>0.2
4-formylamino-antipyrine	0.2	0.2
Diclofenac	0.2	0.2
Ibuprofen	>0.2	>0.2
Ketoprofen	0.02	0.2
Mefenamic acid	0.02	0.2
Naproxen	0.2	>0.2
Oxyphenylbutazone	0.2	>0.2
Phenylbutazone	>0.2	>0.2
Salicylic acid	0.02	>0.2
Corticoids	**SDL**	**LOI**
Betamethasone/Dexamethasone	0.2	>0.2
Flumethasone	0.2	>0.2
Methylprednisolone	>0.2	>0.2
Parasiticide	**SDL**	**LOI**
Leucomalachite green	>0.2	>0.2
Malachite green	0.2	0.2

Other pharmaceuticals	**SDL**	**LOI**
Acetaminophen	>0.2	>0.2
Atorvastatin	0.2	>0.2
Bezafibrate	0.2	>0.2
Carbamazepine	0.02	>0.2
Enalapril	0.02	0.2
Gemfibrozil	0.2	0.2
Irbesartan	0.02	0.2
Lorazepam	0.2	0.2
Olanzapine	0.2	0.2
Pantoprazole	>0.2	>0.2
Pravastatin	0.2	0.2
Valsartan	0.2	0.2
Venlafaxine	0.02	0.02

Table 2. Positives found in 22 feed samples analyzed by UHPLC-QTOF MS.

Positive findings										
Compound	Bovine (n=12)		Rabbit (n=3)		Poultry (n=2)		Goat (n=2)		Pork (n=3)	
	Number of positive findings	Conc (mg kg-1)	Number of positive findings	Conc (mg kg-1)	Number of positive findings	Conc (mg kg-1)	Number of positive findings	Conc (mg kg-1)	Number of positive findings	Conc (mg kg-1)
α-Nandrolone	2		-		2		1		2	
β-Nandrolone	1	0.3	-		-		-		-	
Robenidine	1	<0.2	2	40.4,31.2	-		-		-	
Chlortetracycline	1		-		1		-		-	
Oxytetracycline	-		2		-		-		1	52.4
Tetracycline	1		-		1		-		1	1.7
Doxicycline	-		1		-		-		1	18.7
Lincomycin	1	22.2	2		-		1		3	
Trimethoprim	5		2		1		1	1.2	2	
Florfenicol	2	0.5,0.4	-		-		-		-	
Salicylic acid	3	1.0	3		1		2		2	

Quantification was only made for those compounds and matrices previously tested, and proven to have satisfactory recovery (between 60 and 120%) and RSD (< 15%)

SUPPLEMENTARY INFORMATION

Table S1. Retention time (Rt), ionization mode, elemental composition, exact mass of (de)protonated molecule and elemental composition of the main fragment/adduct ions used for identification of the compounds.

Pharmaceutical	Rt (min)	Ion mode	Elemental composition	$[M+H]^+$ /$[M-H]^-$	Fragment ion 1	Fragment ion 2	Fragment ion 3	Fragment ion 4	Fragment ion 5
Stilbenes									
Dienestrol	11.2	-	C18H18O2	265.1229	C17H14O2	C16H12O2	C6H6O	C15H10O2	C12H12O
Diethylstilbestrol	11.9	-	C18H20O2	267.1385	C14H10O2	C16H16O2	C16H14O2	C15H11O2	C9H8O
Hexestrol	11.3	-	C18H22O2	269.1542	C9H12O	C9H11O	C9H10O	C8H8O	C7H7O
Steroids									
Δ1,4-Androstadiene-3,17-dione (Boldione)	9.6	+	C19H24O2	285.1855	-	-	-	-	-
16-β-hydroxystanozolol	10.5	+	C21H32N2O2	345.2542	C4H4N2	-	-	-	-
α-Boldenone	10.9	+	C19H26O2	287.2011	C19H24O	C10H14	C19H26O2Na	C19H26O2K	C8H8O
α-Nandrolone	11.0	+	C18H26O2	275.2011	C18H24O	C10H12	C18H22	C11H12	C7H8O
Androstenediol (AED)	12.9	+	C19H30O2	291.2324	-	-	-	-	-
β-Boldenone	10.2	+	C19H26O2	287.2011	C19H26O2Na	C19H24O	C10H14	C19H26O2K	C8H8O
β-Nandrolone	10.3	+	C18H26O2	275.2011	C18H24O	C10H12	C18H22	C11H12	C7H8O
Stanozolol	12.1	+	C21H32N2O	329.2593	C7H10	C4H4N2	-	-	-
Resorcylic acid lactones (RALs)									
α-Zeranol	10.5	-	C18H26O5	321.1702	C17H26O3	C17H26O4	C18H24O4	C17H24O2	C15H24O2
β-Zeranol	9.6	-	C18H26O5	321.1702	C17H26O3	C18H24O4	C17H26O4	C17H24O2	C15H24O2
Zearalanone (ZAN)	10.9	-	C18H24O5	319.1545	C18H22O4	C17H24O3	C14H18O3	C12H14O3	C10H12O2
β-Agonists									
Brombuterol	5.6	+	C12H18Br2N2O	364.9864	C12H16N2Br2	C8H8N2Br	C8H8N2Br2	C8H7N2	
Clenbuterol	4.9	+	C12H18Cl2N2O	277.0874	C8H7N2	C12H16N2Cl2	C8H8N2Cl2	C8H5NCl	C8H9N2Cl
Clenpenterol	5.9	+	C13H20Cl2N2O	291.1031	C13H18N2Cl2	C8H8N2Cl2	C8H8N2Cl	C8H5NCl	C7H6NCl
Hydroxymethyl clenbuterol	3.9	+	C12H18Cl2N2O2	293.0824	C12H16N2OCl2	C8H8N2Cl	C8H7N2	C8H8N2Cl2	-
Mabuterol	5.8	+	C13H18ClF3N2O	311.1138	C9H8N2F3Cl	C9H7N2F2Cl	C13H16N2F3Cl	C9H8N2F3	C8H6NF2Cl
Mapenterol	6.8	+	C14H20ClF3N2O	325.1295	C9H8N2F3Cl	C9H7N2F2Cl	C9H8N2F3	C14H18N2F3Cl	C9H6N2FCl
Ractopamine	4.3	+	C18H23NO3	302.1756	C8H8O	C7H6O	C18H21NO2	C10H13NO	C8H9NO
Nitromidazoles									
Chloramphenicol	6.3	+	C11H12Cl2N2O	323.0201	C11H10N2O4Cl2	C8H8N2O2	C11H11NaCl2N2O5	C8H8N	C10H8N2O3Cl2
		-	C11H12N2O5Cl2	321.0045	C10H11N2O4Cl	C9H9NO4	C7H7NO3	C9H7NO3	C10H10N2O5
Dimetridazole (DMZ)	2.8	+	C5H7N3O2	142.0617	C5H10N2O	C4H8N3	C5H9N2	C4H6N2	C5H10N2

Pharmaceutical	Rt (min)	Ion mode	Elemental composition	$[M+H]^+$ /$[M-H]^-$	Fragment ion 1	Fragment ion 2	Fragment ion 3	Fragment ion 4	Fragment ion 5
Furaltadone	2.4	+	C13H16N4O6	325.1148	C5H9NO	C11H13N3O4	C7H13NO	-	-
Hydroxy Dimetridazole (HMMNI)	2.1	+	C5H7N3O3	158.0566	-	-	-	-	-
Hydroxyipronidazole (IPZOH)	4.4	+	C7H11N3O3	186.0879	C7H9N3O2	C7H9N2	C4H5N3O2	C6H6N2	C6H1N
Hydroxymetronidazole (MNZOH)	1.8	+	C6H9N3O4	188.0671	-	-	-	-	-
Ipronidazole (IPZ)	5.4	+	C7H11N3O2	170.0930	-	-	-	-	-
Metronidazole (MNZ)	5.4	+	C6H9N3O3	172.0722	C6H11N2O	C5H9N3O	C6H10N2O	C6H11N3	C5H9N3
Ronidazole (RNZ)	12.1	-	C6H8N4O4	199.0467	C5H6N2O4	C3N2O2	C4H4N3	-	-
Amphenicols									
Amoxicillin	4.2	+	C16H19N3O5S	366.1123	-	-	-	-	-
Ampicillin	4.2	+	C16H19N3O4S	350.1174	C7H7N	C6H9NO2S	C10H7NO2	C8H7N	C4H3NOS
Cefaclor	3.9	+	C15H14N3O4SCl	368.0472	-	-	-	-	-
Cefadroxil	4.9	+	C16H17N3O5S	364.0967	-	-	-	-	-
Cefotaxim	4.0	+	C16H17N5O7S2	456.0647	C14H13N5O5S2	C12H13N5O2S2	C6H4N4OS	C7H6N2OS	C13H13N5O4S2
Ceftriaxone	11.3	+	C18H18N8O7S3	555.0539	-	-	-	-	-
Cefuroxime	9.0	+	C16H16N4O8S	425.0767	-	-	-	-	-
Cephalexin	3.9	+	C16H17N3O4S	348.1018	C6H7NO2S	C6H5NOS	C10H7NO2	C8H7N	C7H7N
Chlortetracycline	6.1	+	C22H23ClN2O8	479.1221	C22H20NO8Cl	C9H5O3Cl	-	-	-
Ciprofloxacin	4.2	+	C17H18N3O3F	332.1410	C17H16N3O2F	C12H7N2O2F	C14H13N2OF	C11H7N2OF	C16H18N3OF
Clarithromycin	10.3	+	C38H69NO13	748.4847	C30H55NO10	C8H15NO2	C29H51NO9	C6H13NO	C5H6O
Cloxacillin	9.4	+	C19H18ClN3O5S	436.0734	C6H9NO2S	C19H17NaClN3O5S	C9H4NOCl	C13H9N2O3Cl	C11H8NO2Cl
Dicloxacillin	10.0	+	C19H17Cl2N3O5S	470.0344	C13H8N2O3Cl2	C19H16NaCl2N3O5S	C14H5N3O3Cl2	C11H7NOCl2	C9H5NCl2
Doxycycline	7.2	+	C22H24N2O8	445.1611	-	-	-	-	-
Enrofloxacin	4.4	+	C19H22FN3O3	360.1723	C18H22N3OF	C14H13N2OF	C19H20N3O2F	-	-
Erythromycin A	9.3	+	C37H67NO13	734.4690	C8H15NO2	C37H65NO12	C6H13NO	C29H53NO10	-
Florfenicol	4.5	+	C12H14Cl2FNO4S	358.0083	C8H9NO2FCl	C6H10NCl	C11H6O3FCl	C8H8NO2F	
Florfenicol amine	1.2	+	C10H14FNO3S	248.0757	-	-	-	-	-
Flumequine	8.1	+	C14H12NO3F	262.0879	C11H4NO2F	C10H4NOF	C14H9NO2F	C14H11NO2F	-
Lincomycin	3.6	+	C18H34N2O6S	407.2216	C8H15N	-	-	-	-
Nalidixic acid	7.7	+	C12H12N2O3	233.0926	C10H6N2O2	C12H10N2O2	C9H6N2O	-	-
Norfloxacin	4.0	+	C16H18N3O3F	320.1410	C16H16N3O2F	C13H13N2OF	C15H18N3OF	C16H15N3O2	C12H7N2O2F
Ofloxacin	3.9	+	C18H20N3O4F	362.1516	C7H20N3O2F	C14H13N2O2F	C18H18N3O3F	C14H9N2O2F	C4H7N
Oxacillin	9.0	+	C19H19N3O5S	402.1124	C13H10N2O3	C6H9NO2S	C19H18NaN3O5S	C14H7N3O3	C11H10N3O3S
Oxolinic acid	6.4	+	C13H11NO5	262.0715	C13H9NO4	C11H5NO4	C10H5NO2	C9H5NO2	C11H4NO4
Oxytetracycline	4.5	+	C22H24N2O9	461.1560	C22H22N2O8	C22H19NO8	C22H21NO9	C22H17NO7	C20H12O8

Pharmaceutical	Rt (min)	Ion mode	Elemental composition	$[M+H]^+$ /$[M-H]^-$	Fragment ion 1	Fragment ion 2	Fragment ion 3	Fragment ion 4	Fragment ion 5
Pefloxacin	3.9	+	C17H20FN3O3	334.1567	C17H18N3O2F	C13H13N2OF	C16H20N3OF	C11H9N2OF	C11H7N2OF
Penicillin G	7.9	+	C16H18N2O4S	335.1065	C10H9NO2	C6H9NO2S	C16H17NaN2O4S	C8H9N2O2S	C8H7NO2S
Pipedimic acid	3.3	+	C14H17N5O3	304.1409	C14H15N5O2	C11H12N4O	C10H6N4O2	C9H8N4O	C8H7N3O
Piperacillin	7.9	+	C23H27N5O7S	518.1709	C14H15N5O7S	C6H10N2O2	C6H9NO2S	C23H26N5NaO7S	C17H18N4O5
Roxythromycin	10.5	+	C41H76N2O15	837.5324	C33H62N2O12	C8H15NO2	C5H6O	C35H60N2O12	C28H45N2O7
Sarafloxacin	4.9	+	C20H17F2N3O3	386.1316	C20H15N3O2F2	C20H11N2F	C19H17N3OF2	C17H20N3O2F	-
Sulfadoxine	4.8	+	C12H14N4O4S	311.0814	C6H5NO2S	C12H13N4O4SNa	C6H5NO	C6H7N3O2	C5H5N3O2
Sulfamethoxazole	4.4	+	C10H11N3O3S	254.0599	C6H5NO2S	C6H5N	C6H5NO	C4H6N2O	-
Tetracycline	4.4	+	C22H24N2O8	445.1611	C22H19NO7	C7H7NO3	-	-	-
Thiamphenicol	3.4	+	C12H15Cl2NO5S	356.0126	C11H8NOCl2	C11H8NOCl	C9H7O	-	-
		-	C12H15Cl2NO5S	353.9970	C8H10O3S	C8H8O	CH4O2S	C11H14NO4SCl	C12H13NO5S
Trimethoprim	3.6	+	C14H18N4O3	291.1457	C12H12N4O3	C14H15NO2	C12H13N4O	C13H14N4O3	C13H12N4O2
Avermectins									
Abamectine B1a	15.4	+	C48H71O14Na	895.4820	C48H72O14	-	-	-	-
Doramectine	16.8	+	C50H74O14	899.5157	C50H74O14Na	C50H74O14K	-	-	-
Emamectin B1a	13.5	+	C49H75NO13	886.5317	C7H11NO	C49H75NO13Na	C12H13	C19H25O3	-
Eprinomectin	14.9	+	C50H75NO14	914.5266	C50H75NO14Na	C6H9NO	C15H23NO5	C9H15NO3	C8H11NO2
Ivermectin	16.0	+	C48H74O14	875.5157	C48H73O14Na	-	-	-	-
Levamisole	2.8	+	C11H12N2S	205.0799	C10H11NS	C7H6	C7H6S	C10H8	-
Moxidectin	15.6	+	C37H53NO8	640.3849	C29H37NO6	C30H41NO7	C37H53NO8Na	C37H53NO8K	C29H41NO6
Coccidiostats									
Maduramicine	15.5	+	C47H79O17Na	939.5293	C46H77O14Na	C42H77O18Na	C37H56O8	-	-
Monensin	15.1	+	C36H62O11	671.4370	C36H61O11Na	C36H61O11K	C28H44O5	C36H59O10Na	-
		-	C36H62O11	669.4214	C35H58O10	C11H20O2	C5H10O2	C4H8O2	-
Narasin	16.1	+	C43H72O11	765.5153	C13H20O3	C23H32O4	C43H72O11Na	C42H68O10	C42H66O9
		-	C43H72O11	763.4996	C23H36O6	C20H36O5	C19H29O5	C14H24O4	C13H24O2
Robenidine	10.8	+	C15H13Cl2N5	334.0626	C8H4N3Cl	C7H7N2Cl	C7H5Cl	C8H7N4Cl	C7H6NCl
Salinomycin	15.7	+	C42H70O11	751.4996	C42H70O11Na	C42H70O11K	C42H68O10	C42H66O9	-
		-	C42H70O11	749.4840	C19H30O5	C35H60O11	C23H36O6	C19H34O5	-
Sedatives									
Acepromazine	8.8	+	C19H22N2OS	327.1531	C5H11N	C15H12NS	C13H9NS	C17H15NOS	C15H11NOS
Alprazolam	9.6	+	C17H13ClN4	309.0907	C16H11N3Cl	C17H13N4	C15H4N4	C14H8N2	C8H5N2Cl
Azaperol	5.0	+	C19H24FN3O	330.1982	C10H9F	C7H8N2	C19H22N3F	C12H14NF	C9H12N2
Azaperone	5.6	+	C19H22FN3O	328.1825	C10H9OF	C7H3OF	C7H10N2	C9H10N2	-
Carazolol	6.0	+	C18H22N2O2	299.1760	C15H11NO	C12H9NO	C6H13NO	C3H7NO	C13H9NO

Pharmaceutical	Rt (min)	Ion mode	Elemental composition	$[M+H]^+$ /$[M-H]^-$	Fragment ion 1	Fragment ion 2	Fragment ion 3	Fragment ion 4	Fragment ion 5
Chlorpromazine	10.1	+	C17H19ClN2S	319.1036	C13H8NCl	C5H11N	C15H12NSCl	C13H8NSCl	C13H8NS
Propionilpromazine (combelen)	9.8	+	C20H24N2OS	341.1688	C16H13NOS	C5H11N	C16H13NO	C18H17NOS	C3H7N
Non steroidal anti-inflammatory (NSAIDs)									
4-Acetylamino-antipyrine	3.6	+	C13H15N3O2	246.1242	C13H15N3O2	C13H13N3O	C11H13N3O	C7H5N	C10H10N2
4-amino-antipyrine	3.5	+	C11H13N3O	204.1137	C4H6N2	C7H5N	C4H6N2	C6H7N	C6H7N
4-formylamino-antipyrine	3.5	+	C12H13N3O2	232.1086	C12H12N3O2Na	C12H12N3O2K	C7H5N	C4H6N2	C6H7NO
Diclofenac	12.1	+	C14H11Cl2NO2	296.0245	C14H10NaCl2NO2	C14H9NOCl2	C13H8NCl	C13H9NCl	-
		-	C14H11Cl2NO2	294.0089	C13H9NCl2	C13H8NCl	-	-	-
Ibuprofen	12.4	-	C13H18O2	205.1229	C12H16	-	-	-	-
Ketoprofen	10.1	+	C16H14O3	255.1021	C16H13NaO3	C15H12O	C14H9O	C7H4O	-
		-	C16H14O3	253.0865	C15H12O	C13H8O2	-	-	-
Mefenamic acid	13.2	+	C15H15NO2	242.1181	C15H13NO	C14H10NO	C13H9N	-	-
Naproxen	10.4	+	C14H14O3	231.1021	C13H12O	C12H9O	C14H13NaO3	C12H8	C11H8
Oxyphenylbutazone	9.8	+	C19H20N2O3	325.1552	C19H19N2O3Na	C12H13NO2	C8H5NO2	C7H5NO	C11H13N
Phenylbutazone	11.4	+	C19H20N2O2	309.1603	C12H13NO	C11H13N	C7H5NO	C8H5NO	C6H5N
Salicylic acid	5.2	-	C7H6O3	137.0239	C6H4O	-	-	-	-
Corticoids									
Betamethasone o Dexamethasone	9.9	+	C22H29FO5	393.2077	C22H28O5	C22H26O4	C22H24O3	C22H22O2	C21H22O2
Flumethasone	9.5	+	C22H28F2O5	411.1983	C17H16O2	C14H15O2F	C8H8O	C9H10O	-
Methylprednisolone	9.9	+	C22H30O5	375.2171	C13H12O	C11H12O	C20H22O	C17H16O	C12H12O
Parasiticide									
Leucomalachite green	12.6	+	C23H26N2	331.2171	C14H13N	C16H18N2	C13H9N	C22H23N2	C17H20N2
Malachite green	9.7	+	C23H25ClN2	365.1785	C23H24N2	C22H20N2	C15H13N	C17H17N2	
Other pharmaceuticals									
Acetaminophen	2.2	+	C8H9NO2	152.0711	C6H7NO	C6H5N	C6H4O	C8H7NO	C8H7NO2
Atorvastatin	11.9	+	C33H35FN2O5	559.2608	C26H30NO4F	C20H18NF	C33H34NaFN2O5	C27H28NO5F	C19H14NF
		-	C33H35FN2O5	557.2452	C29H25N2O2F	C26H21N2OF	-	-	-
Bezafibrate	10.6	+	C19H20ClNO4	362.1159	C18H18NO2Cl	C15H14NO2Cl	C11H12O	C7H3OCl	C8H8O
		-	C19H20ClNO4	360.1003	C15H12NO2Cl	C7H4NOCl	C3H2NO2	-	-
Carbamazepine	8.1	+	C15H12N2O	237.1028	C14H10N	C14H11N	C13H8N	-	-
		-	C15H12N2O	235.0871	C14H12N	C14H11N	C14H10N	C13H9N	-
Enalapril	7.8	+	C20H28N2O5	377.2076	C14H19NO2	C14H19NO2	C11H13N	C17H22N2O3	C9H11N

Pharmaceutical	Rt (min)	Ion mode	Elemental composition	$[M+H]^+$ /$[M-H]^-$	Fragment ion 1	Fragment ion 2	Fragment ion 3	Fragment ion 4	Fragment ion 5
Gemfibrozil	13.3	+	C15H22O3	251.1647	C15H21NaO3	C7H12O2	C15H20O2	-	-
	13.3	-	C15H22O3	249.1491	C8H8O	-	-	-	-
Irbesartan	10.6	+	C25H28N6O	429.2403	C14H10N2	C11H18N2O	C13H9N	C14H9N	-
		-	C25H28N6O	427.2246	C11H18N2O	C25H28N4O	-	-	-
Lorazepam	9.6	+	C15H10Cl2N2O2	321.0198	C14H8N2Cl2	C13H9N2Cl	C15H9NaCl2N2O2	C15H8N2OCl2	C14H7N2Cl
Olanzapine	3.7	+	C17H20N4S	313.1487	C14H13N3S	C12H8N2S	C16H15N3S	C11H5N2S	C11H7NS
Omeprazole	7.8	+	C17H19N3O3S	346.1225	C9H11NO2S	C9H11NO	C8H8N2O	C9H9NOS	C8H6NOS
Pantoprazole	8.1	+	C16H15F2N3O4S	384.0830	C7H7NO2	C8H9NO3S	C8H10NO2	-	-
Pravastatin	9.8	+	C23H36O7	425.2539	C23H35NaO7	C20H22O4	-	-	-
		-	C23H36O7	423.2383	C5H10O2	C18H24O4	C11H12	-	-
Valsartan	11.0	+	C24H29N5O3	436.2349	C24H28N5O3Na	C18H19N5	C14H10N4	C24H27N5O2	C24H29N3O3
		-	C24H29N5O3	434.2192	C14H12	C14H11	C19H21N5O2	-	-
Venlafaxine	7.0	+	C17H27NO2	278.2120	C17H25NO	C8H8O	C15H18O	-	-

Molecular formulae of the fragment ions differs ±1 hydrogen from the real one, depending on the ionization mode

Table S2. Validation results obtained after analysis of ten animal feed samples spiked at 0.02 and 0.2 mg kg-1. Screening detection limits (SDL) and limits of identification (LOI) for each matrix, and SDL of the method (all in mg kg-1).

Pharmaceutical	Poultry (n=2)		Bovine (n=2)		Rabbit (n=2)		Pork (n=2)		Goat (n=2)	
	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI
Stilbenes										
Dienestrol	0.2	0.2	0.2	-	-	-	0.2	-	-	-
Diethylstilbestrol	-	-	-	-	-	-	-	-	-	-
Hexestrol	0.2	0.2	-	-	0.2	-	0.2	0.2	-	-
Steroids										
Δ1,4-Androstadiene-3,17-dione (Boldione)[a]	0.02	-	0.2	-	0.2	-	-	-	-	-
16-β-hydroxystanozolol[b]	-	-	-	-	-	-	-	-	-	-
α-Boldenone	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.2
α-Nandrolone	0.02	0.02	0.02	0.2	0.02	-	0.02	0.02	0.02	0.02
Androstenediol (AED)[a]	0.02	-	0.02	-	-	-	0.02	-	0.02	-
β-Boldenone	0.02	0.02	0.02	-	0.02	0.2	0.02	0.02	0.2	0.2
β-Nandrolone	0.02	0.2	0.02	0.2	0.02	0.02	0.2	0.2	0.02	0.02
Stanozolol	0.02	0.2	0.02	-	0.02	0.02	0.02	-	0.02	-
RALs										
α-Zeranol	0.02	0.2	0.2	0.2	-	-	0.02	0.2	0.2	0.2
β-Zeranol	0.02	0.2	0.2	0.2	0.02	0.2	0.02	0.2	0.2	-
Zearalanone (ZAN)	-	-	-	-	0.2	-	-	-	-	-
β-Agonists										
Brombuterol	0.02	0.02	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02
Clenbuterol	0.02	0.02	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02
Clenpenterol	0.02	0.02	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02
Hydroxymethyl clenbuterol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mabuterol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Mapenterol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Ractopamine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.2
Nitromidazoles										
Chloramphenicol	0.02	0.2	0.02	0.2	0.02	0.2	0.02	0.2	0.02	0.2
Dimetridazole (DMZ)	0.02	-	0.02	-	0.02	0.2	0.02	0.2	0.02	0.02
Furaltadone	0.02	0.2	0.02	-	0.2	0.2	0.02	0.2	0.02	0.2
Hydroxy Dimetridazole (HMMNI) [a]	-	-	0.02	-	-	-	-	-	-	-
Hydroxyipronidazole (IPZOH)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Hydroxymetronidazole (MNZOH)[a]	0.2	-	0.2	-	0.2	-	0.2	-	-	-
Ipronidazole (IPZ)[a]	0.02	-	0.2	-	0.2	-	0.02	-	0.02	-
Metronidazole (MNZ)	-	-	-	-	0.02	-	-	-	-	-
Ronidazole (RNZ)	0.2	-	0.2	-	-	-	0.2	-	-	-
Amphenicols										
Amoxicillin[a]	-	-	-	-	-	-	-	-	-	-
Ampicillin	0.02	0.2	0.2	0.2	0.2	-	0.2	0.2	0.2	0.2
Cefaclor[a]	-	-	-	-	-	-	-	-	-	-
Cefadroxil[a]	-	-	-	-	-	-	-	-	-	-
Cefotaxim	0.02	0.2	0.2	0.2	0.2	0.2	0.02	0.02	0.2	0.2
Ceftriaxone[a]	-	-	0.2	-	-	-	-	-	-	-
Cefuroxime[a]	-	-	0.2	-	0.2	-	-	-	-	-
Cephalexin	-	-	-	-	-	-	-	-	-	-
Chlortetracycline	-	-	0.2	0.2	0.2	-	0.2	-	0.2	-
Ciprofloxacin	0.02	0.2	0.2	0.2	0.2	0.2	0.02	0.02	0.2	0.2
Clarithromycin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Pharmaceutical	Poultry (n=2)		Bovine (n=2)		Rabbit (n=2)		Pork (n=2)		Goat (n=2)	
	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI
Cloxacillin	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.2	0.2
Dicloxacillin	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-
Doxycycline[a]	-	-	-	-	0.02	-	0.02	-	0.02	-
Enrofloxacin	0.02	0.2	0.02	0.2	0.2	0.2	0.02	0.2	0.02	0.2
Erythromycin A	0.2	0.2	0.2	0.2	0.2	-	0.2	-	0.2	-
Florfenicol	0.2	-	0.2	0.2	0.02	0.02	0.2	0.2	0.2	0.2
Florfenicol amine[a]	0.2	-	0.2	-	-	-	0.2	-	-	-
Flumequine	0.02	0.2	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Lincomycin[b]	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Nalidixic acid	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Norfloxacin	0.02	0.2	0.2	0.2	0.02	0.2	0.02	0.02	0.2	0.2
Ofloxacin	0.02	0.2	0.2	0.2	0.02	0.2	0.02	0.02	0.02	0.2
Oxacillin	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-
Oxolinic acid	0.02	0.02	0.2	0.2	0.02	0.2	0.02	0.02	0.02	0.2
Oxytetracycline	-	-	0.2	0.2	0.02	0.02	0.02	0.02	-	-
Pefloxacin	0.02	0.02	0.02	0.2	0.02	0.2	0.02	0.2	0.02	0.2
Penicillin G	0.2	0.2	0.02	0.2	0.02	0.2	0.2	0.2	0.2	0.2
Pipedimic acid	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.02	0.2
Piperacillin	0.2	0.2	0.02	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Roxythromycin	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Sarafloxacin	0.02	0.2	0.02	0.2	0.2	-	0.02	0.2	0.2	0.2
Sulfadoxine	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Sulfamethoxazole	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.2	0.02	0.2
Tetracycline	-	-	0.2	-	-	-	0.02	0.02	-	-
Thiamphenicol	0.02	-	0.2	-	0.02	-	0.02	0.2	0.02	-
Trimethoprim	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Avermectins										
Abamectine B1a[b]	-	-	-	-	-	-	-	-	-	-
Doramectine	-	-	-	-	-	-	-	-	-	-
Emamectin B1a	0.02	0.02	0.02	0.2	0.02	0.02	0.2	0.2	0.02	0.2
Eprinomectin	0.2	-	0.2	-	0.2	-	0.2	0.2	0.2	0.2
Ivermectin[b]	0.2	-	-	-	-	-	0.2	-	-	-
Levamisole	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.2
Moxidectin	0.2	0.2	0.2	-	-	-	0.2	0.2	0.2	0.2
Coccidiostats										
Maduramicine	0.02	0.2	0.02	0.2	0.02	0.2	0.02	0.2	0.02	0.2
Monensin	0.02	0.02	0.02	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Narasin	0.02	0.2	0.02	0.2	0.02	0.2	0.02	-	0.02	0.2
Robenidine	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.2
Salinomycin	0.02	0.02	0.2	0.2	0.2	0.2	0.2	-	0.2	-
Sedatives										
Acepromazine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.2
Alprazolam	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.2
Azaperol	0.02	0.02	0.02	0.02	0.02	0.2	0.02	0.02	0.02	-
Azaperone	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Carazolol	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Chlorpromazine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Propionilpromazine (combelen)	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
NSAIDs										
4-acetylamino-antipyrine	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
4-amino-antipyrine	0.2	0.2	0.02	-	0.2	-	0.02	-	-	-

Pharmaceutical	Poultry (n=2)		Bovine (n=2)		Rabbit (n=2)		Pork (n=2)		Goat (n=2)	
	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI	SDL	LOI
4-formylamino-antipyrine	0.02	0.2	0.02	0.2	0.2	0.2	0.02	0.02	0.02	0.02
Diclofenac	0.2	0.2	0.2	0.2	0.2	0.2	0.02	0.02	0.2	0.2
Ibuprofen[b]	-	-	-	-	-	-	0.2	0.2	-	-
Ketoprofen	0.02	0.02	0.02	0.2	0.02	0.2	0.02	0.02	0.02	0.2
Mefenamic acid	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Naproxen	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.02	-
Oxyphenylbutazone	0.02	0.2	0.2	0.2	0.2	-	0.02	0.2	0.2	-
Phenylbutazone	0.2	0.2	0.2	0.2	0.2	-	0.02	0.2	-	-
Salicylic acid[b]	0.02	-	0.02	-	0.02	-	0.02	-	0.02	0.2
Corticoids										
Betamethasone/Dexamethasone	0.2	0.2	0.2	0.2	0.2	-	0.2	0.2	0.2	0.2
Flumethasone	0.2	0.2	0.2	-	0.2	0.2	0.2	0.2	0.2	-
Methylprednisolone	-	-	0.2	-	-	-	0.2	0.2	-	-
Parasiticide										
Leucomalachite green	0.02	0.02	-	-	-	-	0.2	0.2	0.2	0.2
Malachite green	0.02	0.02	0.02	0.02	0.02	0.2	0.2	0.2	0.02	0.02
Other pharmaceuticals										
Acetaminophen	0.2	-	0.2	-	0.2	-	0.2	-	-	-
Atorvastatin	0.2	0.2	0.2	0.2	0.2	-	0.2	0.2	0.2	0.2
Bezafibrate	0.02	0.2	0.02	0.2	0.2	0.2	0.02	-	0.02	-
Carbamazepine	0.02	0.02	0.02	0.2	0.02	0.02	0.02	-	0.02	0.02
Enalapril	0.02	0.02	0.02	0.2	0.02	0.2	0.02	0.02	0.02	0.02
Gemfibrozil	0.02	0.02	0.2	0.2	0.2[c]	0.2[c]	0.2	0.2	0.2[c]	0.2[c]
Irbesartan	0.02	0.02	0.02	0.2	0.02	0.02	0.02	0.02	0.02	0.02
Lorazepam	0.2	0.2	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Olanzapine	0.02	0.02	0.2	0.2	0.2	0.2	0.02	0.02	0.2	0.2
Omeprazole	-	-	-	-	-	-	-	-	-	-
Pantoprazole	0.2	0.2	0.2	0.2	-	-	0.2	-	-	-
Pravastatin	0.02	0.2	0.2	0.2	0.02	0.2	0.02	0.02	0.02	0.2
Valsartan	0.02	0.02	0.02	0.02	0.2	0.2	0.02	0.02	0.02	0.02
Venlafaxine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

[a] Compound without fragment ions
[b] Compound with only one fragment ion
[c] Found in negative ionization mode

Table S3. Recovery experiments for the compounds detected in feed samples.

Compound	Ionization mode	Recovery (RSD) (both in %)						Isotopically-labelled internal standard (ILIS)
		Bovine (n=3)		Poultry (n=3)		Pork (n=3)		
		0.2 mg kg-1	2 mg kg-1	0.2 mg kg-1	2 mg kg-1	0.2 mg kg-1	2 mg kg-1	
β-Nandrolone	+	94 (14)	144 (3)	76 (15)	123 (3)	93 (5)	110 (4)	Fenilbutazone-d_{10}
Robenidine	+	93 (6)	94 (3)	61 (3)	90 (3)	70 (1)	98 (6)	Robenidine-d_8
Chlortetracycline	+	-	-	-	-	-	81 (22)	Amphetamine-d_6
Oxytetracycline	+	-	-	-	-	-	108 (9)	Amphetamine-d_6
Tetracycline	+	-	-	-	-	-	101 (4)	Amphetamine-d_6
Doxicycline	+	-	-	-	-	-	115 (11)	Amphetamine-d_6
Lincomycin	+	105 (1)	121 (2)	92 (0)	104 (2)	-	-	Carbamazepine epoxide-d_{10}
Trimethoprim	+	57 (5)	120 (2)	51 (0)	96 (6)	33 (7)	85 (2)	Fenilbutazone-d_{10}
Florfenicol	+	73 (8)	58 (4)	71 (9)	98 (2)	59 (10)	81 (10)	Benzoylecgonine-d_3
Acid salicylic	-	-	108 (10)	-	39 (19)	-	132 (7)	4,4'dinitrocarbanilide-d_8

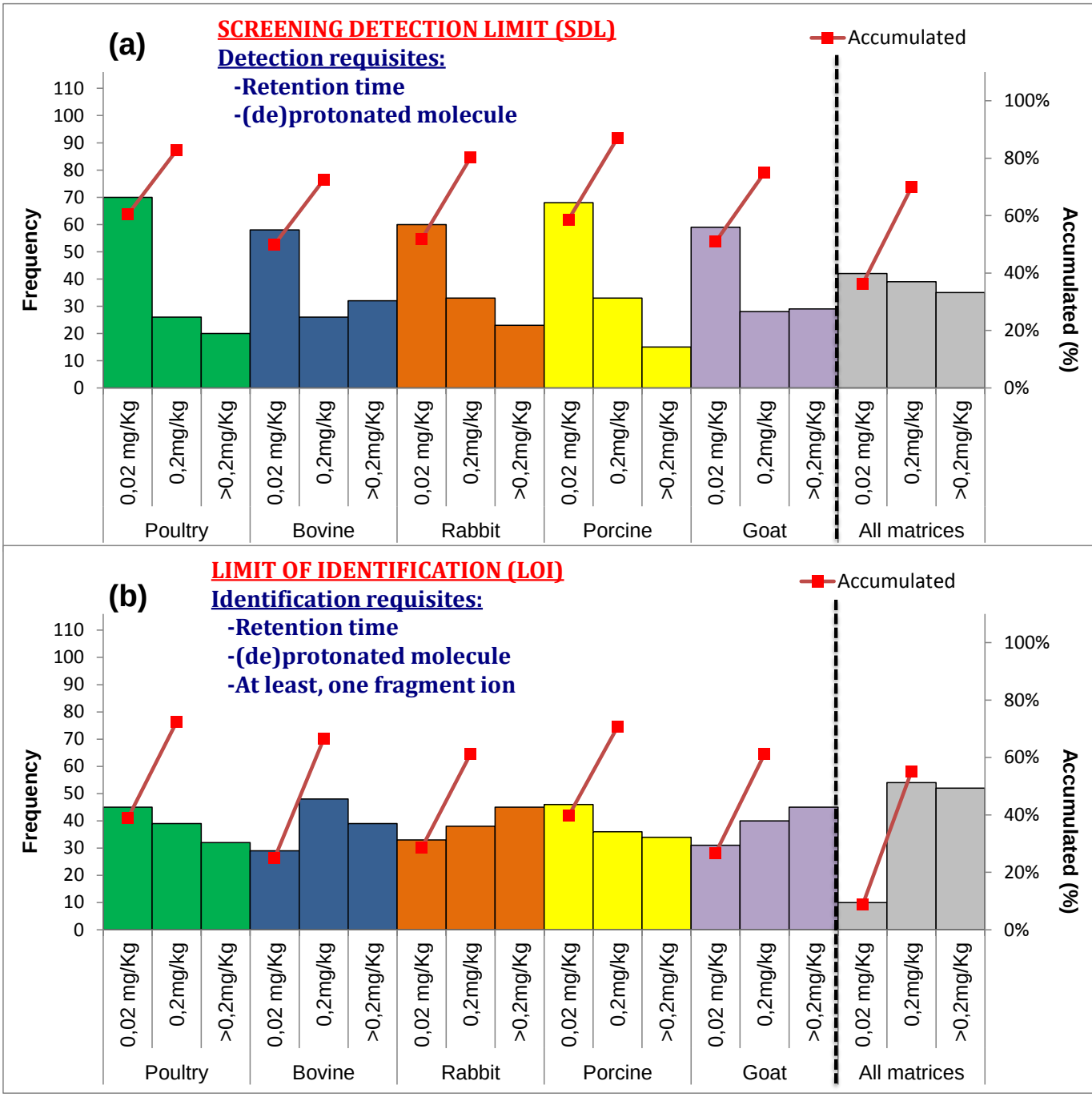


Figure S1. Number of analytes (a) detected and (b) identified at 0.02 and 0.2 mg kg-1 in each feed matrix.

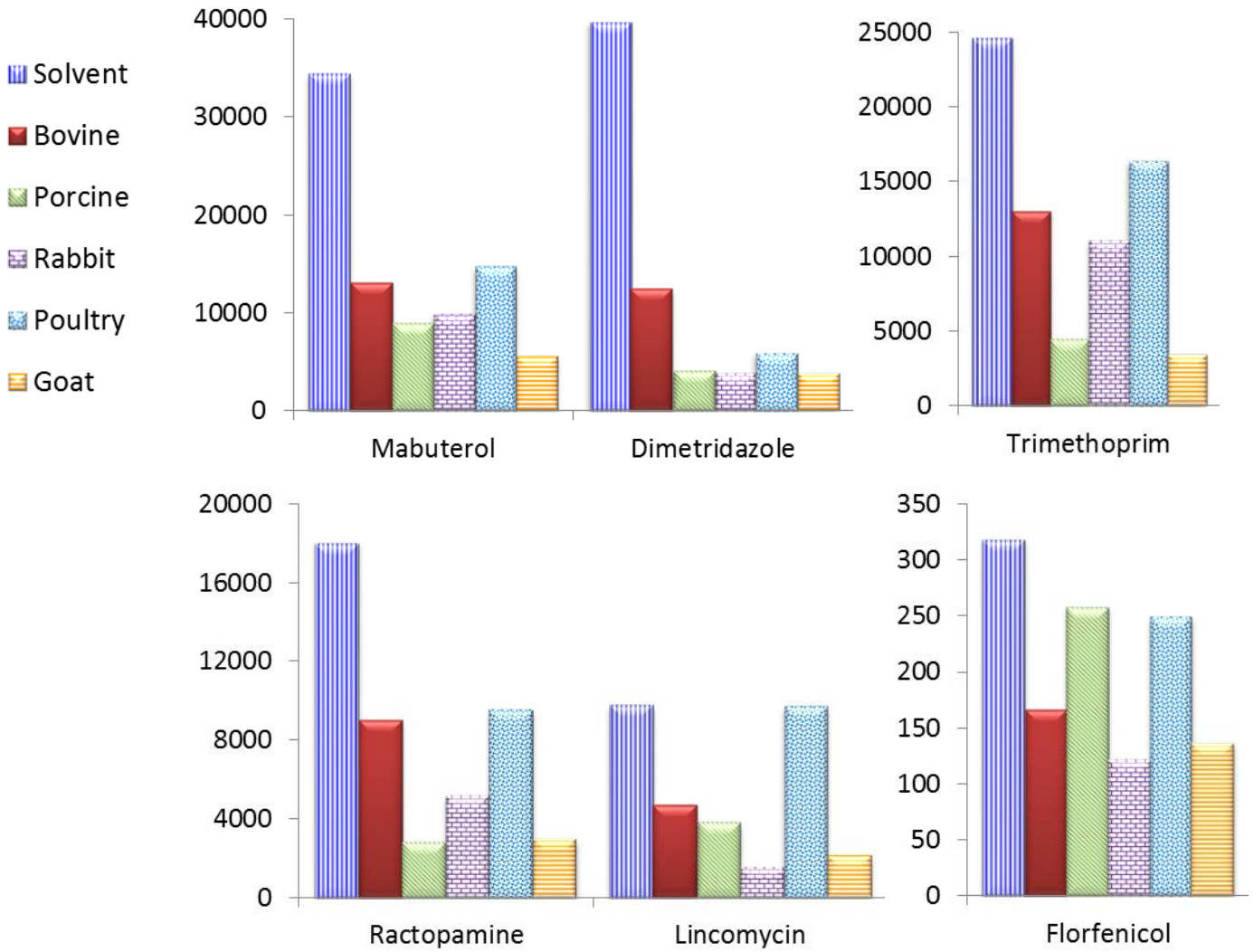


Figure S2. Absolute response for six pharmaceuticals in solvent and in five feed matrices spiked at 0.2 mg kg-1.

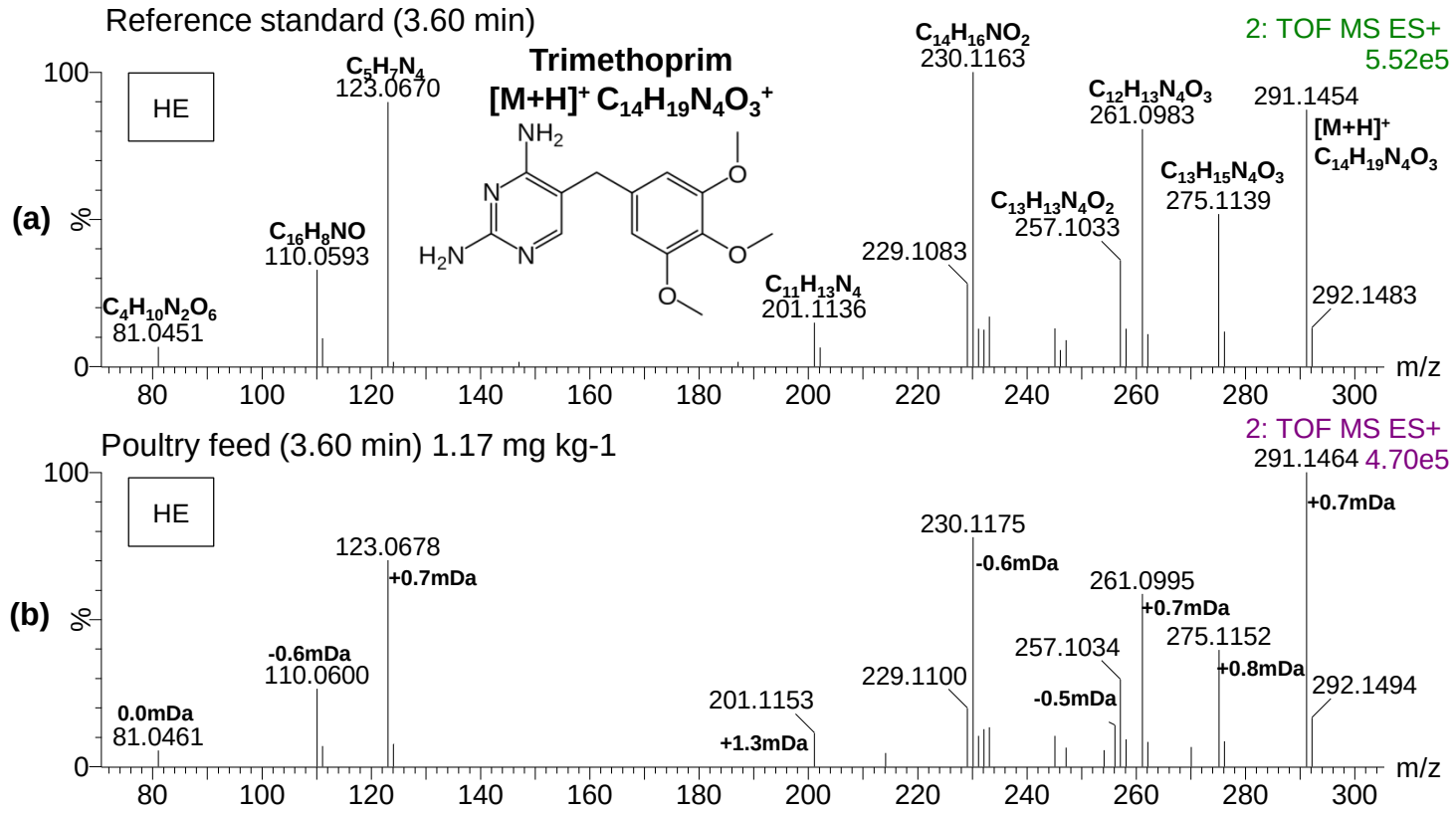


Figure S3. HE mass spectra of trimethoprim in (a) reference standard and (b) poultry feed sample.